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(54) **Preparation of non-crystalline and crystalline dihydrate forms of azithromycin**

Herstellung von nicht-kristallinen und kristallinen Dihydratformen von Azithromycin

Preparation des formes non-crystalline et crystalline dihydrate de l' azithromycin

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(73) Proprietor: **Astur-Pharma, S.A.**
28033 Madrid (ES)

(72) Inventors:
- **Bayod Jasanada, Miguel Santos**
  33192 Silvota (Asturias) (ES)
- **Llorente Garcia, Isidro**
  33192 Silvota (Asturias) (ES)
- **Fernandez Mari, Felix**
  33192 Silvota (Asturias) (ES)

(74) Representative: **KOHLER SCHMID + PARTNER**
Patentanwälte GbR,
Ruppmannstrasse 27
70565 Stuttgart (DE)

(56) References cited:
EP-A- 0 298 650
EP-A- 0 827 965
WO-A-94/26758
WO-A-99/58541
US-A- 4 517 359

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Description

BACKGROUND OF THE INVENTION

1. Field of the Invention.

[0001] Azithromycin is the USAN generic name of the azalide 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A, which systematic name is 1-oxa-6-azacyclopentadecan-15-one,13-((2,6-dideoxy-3-C-methyl-1-3-O-methyl-alpha-L-ribohexopyranosyl)-oxy)-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-((3,4,6-trideoxy-3-(dimethyl-amino)-beta-D-xylo-hexopyranosyl)oxy). It is a semisynthetic macrolide that shows an excellent antimicrobial activity against grampositive and some cases of gram-negative bacteria (H.A. Kirst, G.D. Sides, *Antimicrob. Agents. Chemother.* **1989,** *33,* 1419-1422). Clinical use of this macrolide is broadening its application to the treatment of opportunistic infections (F. Lecomte, *Rev. Med. Interne* **1998,** *19(4)*, 255-61; S. Alvarez-Elcoro, *Mayo Clin. Proc.* **1999,** *74(6)*, 613-34; J. Schater, *Lancet,* **1999,** *354(9179)*, 630-35).

2. Description of the Prior Art.

[0002] **Figure 1** shows the different synthetic routes to azithromycin **1**. The names of the intermediates displayed in **Figure 1** are gathered in the following table.

Intermediate	Name
1	Azithromycin
2	Erythromycin A oxime
3	6,9-iminoether
4	9,11-iminoether
5	Azaerythromycin A
6	Azaerythromycin 11,12-hydrogenorthoborate
7	Azithromycin 11,12-hydrogenorthoborate

[0003] The following table summarizes the patents, articles, authors and applicants that describe the different synthetic paths (**A, B, C, D, E**) towards azithromycin **1**.

Route	Patents	Articles	Author	Applicant
A	• US 4,328,334 • US 4,517,359	• J. Chem. Soc. Perkin Trans I, 1986, 1881 • J. Chem. Res., 1988, 132 • Idem miniprint., 1988, 1239	S. Djokic	PLIVA
B	• US 4,474,768		G.M. Bright	PFIZER
C	• US 5,686,587 • EP 0,699,207 • ES 2,104,386		B.V. Yang	PFIZER
D	• US 5,869,629 • EP 0,827,965 • ES 2,122,905	• J.Org.Chem, 1997, 62, (21), 7479 – 7481 • Magn. Reson. Chem, 1998, 36, 217-225	M. Bayod	ASTUR PHARMA
E	• EP 0,879,823		W. Heggie J.Diago	HOVIONE BIOCHEMIE
F	• WO 99/58541			

The structural elucidation studies carried out with azithromycin **1** have shown the existence of two different crystalline forms: hygroscopic monohydrate and non-hygroscopic dihydrate, being the latter preferred for manufacturing formulations used in therapeutical treatments, as it is described in **EP 0,298,650**.

[0004] Azithromycin dihydrate is easily distinguishable from hygroscopic azithromycin by means of the following differentiative assays:

a) The dihydrate form keeps its percentile water content constant at values (4.5-5%) which are very close to the theoretical value (4.6%).
b) The differential calorimetry analysis (DSC) of azithromycin dihydrate reveals the presence of a single endotherm which may vary between 115 and 135 °C, with an energy absorbed during the process which ranges from 27 to 34 cal/g.
c) Each crystalline form presents its own characteristic X-Ray Diffraction spectrum.
d) The infrared spectra in KBr of both crystalline forms present clear differences:

azithromycin dihydrate	azithromycin monohydrate
ν (cm^{-1})	ν (cm^{-1})
3560 and 3496 (2 sharp bands)	3500 (wide band)
1344	Does not present any
1282 and 1268 (2 sharp bands)	1280
1083	Does not present any

[0005] Two other synthesis, affording azithromycin **1** as a form that should differ from the crystalline ones previously mentioned, have also been described. In these cases, azithromycin is obtained by simple evaporation to dryness. However, in these documents there is no reference to the crystalline state of the azithromycin thus obtained.

Patent	Applicant (Author)	Priority	Procedure
• WO 94/26758 a) US 5,686,587 b) EP 0,699,207 c) ES 2,104,386	PFIZER (B.V. Yang)	May 19,1993	Methylene chloride evaporation
• BE 892,357 • US 4,517,359	PLIVA (S.Djokic)	Mar. 3, 1981	Chloroform evaporation

[0006] In the following table are summarized the different procedures for the preparation of both crystalline forms of azithromycin **1**.

Crystalline form	Patent	Applicant (Author)	Priority	Procedure
HYGROSCOPIC MONOHYDRATE	• EP 0,101,186 • US 4,474,768	PFIZER (G.M. Bright)	July 19, 1982	Recrystallization from ethanol/water
HYGROSCOPIC MONOHYDRATE	• EP 0,298,650	PFIZER (D. Allen)	July 9, 1997	Recrystallization from ethanol/water
NON-HYGROSCOPIC DIHYDRATE	• EP 0,298,650 • WO 89/00576 • ES 2,038,756	PFIZER (D. Allen)	July 9, 1997	Recrystallization from THF / petroleum ether/ water
NON-HYGROSCOPIC DIHYDRATE	• CN 1,093,370 (Chem. Abs. 29525 q, 124,1996)	Faming Zhuanli.... (Q. Song)	Dec. 10, 1993	• Recrystallization from acetone/water • Recrystallization from other solvents (methanol, DMF, acetonitrile, dioxane, ...) and water
NON-HYGROSCOPIC DIHYDRATE	• EC 95-1389	CHEMO-TECNICA SINTYAL	May, 1995	Recrystallization from acetone/ water
NON-HYGROSCOPIC DIHYDRATE	• EP 0,827,965 • ES 2,122,905 • US 5,869,629	ASTUR PHARMA (M.Bayod)	July 11, 1996	Recrystallization from acetone/ water
NON-HYGROSCOPIC DIHYDRATE	• EP 0,941,999	HOVIONE (W.Heggie)	Mar. 13, 1998	Precipitation from a base neutralized acid solution of azithromycin in acetone/ water

Crystalline form	Article	Author	Date	Procedure
NON-HYGROSCOPIC DIHYDRATE	• J. Chem. Res., 1988, 132 • idem miniprint., 1988, 1239,	S.Djokic (PLIVA)	May, 1988 (received June 4, 1987)	Two recrystallizations: 1. Precipitation from a base neutralized acid solution of azithromycin in acetone/ water. 2. From ethyl ether.
NON-HYGROSCOPIC DIHYDRATE	• J. Org. Chem, 1997, 62, (21), 7479 – 7481	M.Bayod (ASTUR-PHARMA)	Nov., 1997 (received May 1, 1997)	Recrystallization from acetone/ water
HYGROSCOPIC MONOHYDRATE	• J. Org. Chem, 1997, 62, (21), 7479 – 7481	M.Bayod (ASTUR PHARMA)	Nov., 1997 (received May 1, 1997)	Recrystallization from ethanol/water

[0007] The patent WO 99/58541 claims azithromycin in a stable anhydrous form , or in the form of a solvate with non-halogenated solvent with the exception of water. The product is obtained by removal of the non-halogenated solvent from the reaction mixture by evaporation to dryness until crystallization or precipitation occurs, or by extraction and precipitation or crystallization. As in this patent there is no clear reference to the crystallinity of the product, the referred patent has not been included in any of the previous tables.

DESCRIPTION OF THE INVENTION.

[0008]

- First, the present invention provides a procedure for the preparation of non-crystalline azithromycin by means of lyophilization of solutions of azithromycin monohydrate in aliphatic alcohols or cyclic ethers, as *tert*-butanol (2-methyl-2-propanol) or 1,4-dioxane.
- Secondly, the present invention describes the characterization of non-crystalline azithromycin and its unambiguous differentiation from the crystalline forms (dihydrate and monohydrate) using the following techniques:

- ✓ Infrared Spectroscopy
- ✓ Differential Scan Calorimetry (DSC)
- ✓ X-Ray Diffraction
- ✓ Hygroscopicity
- ✓ Crystallinity test by means of polarized light microscopy

[0009] The procedure which is the object of the present invention is advantageous over previously described methods, essentially at industrial scale because the lyophilization is a technique that guarantees excellent results concerning homogeneity, purity and consistency of analytical data of different batches.

[0010] The differences observed between crystalline azithromycin dihydrate and its non-crystalline form, using the techniques previously mentioned, are shown below:

1. Infrared Spectra (KBr), recorded on a FT-IR Nicolet ® Impact 410 Instrument, of both azithromycin forms are clearly different. Fig. 2 reproduces the spectra which most significative bands are summarized in the following table:

Crystalline azithromycin dihydrate	Non-crystalline azithromycin
ν (cm^{-1})	ν (cm^{-1})
3561 and 3496 (2 sharp bands)	3500 (wide band)
1344	Does not present any
1282, 1269 and 1251 (3 sharp bands)	1280 and 1257 (2 sharp bands)
1083	Does not present any

2. DSC. In **Fig. 3** are shown the thermograms obtained scanning between 20 and 300°C, under nitrogen with a heating rate of 5°C / min. The thermogram of the non-crystalline form does not present any melting peak, what clearly differentiates it from the one corresponding to crystalline azithromycin dihydrate.
3. X-Ray Diffraction Spectra were recorded on a Philips® PW1710 diffractometer. As the spectrum corresponding to non-crystalline azithromycin (**Fig. 4**) is characterized by the absence of defined maxima, this solid is considered to be amorphous.
4. Hygroscopicity. Two different samples of non-crystalline azithromycin containing 3% water were kept under an atmosphere over 75% relative humidity. After 8 hours, water content in the first sample was 5.3%, while the second one contained 9.9% water after 72 hours. Non-crystalline azithromycin is thus moderately hygroscopic.
5. Crystallinity tests (polarized light microscopy) carried out with non-crystalline azithromycin were negative, as its particles do not show birefringence.

EXPERIMENTAL PART

[0011]

- **Preparation of 9-deoxo-9a-aza-11,12-desoxy-9a-homoerythromycin A 11,12-hydrogenorthoborate.**

89 g of 9-deoxo-6-desoxy-6,9-epoxy-9,9a-dihydro-9a-aza-homoerythromycin A are dissolved in 450 ml of methanol and cooled down between -5° and -10 °C. While keeping the temperature in the specified interval 16 portions of 2.2 g each of sodium borohydride are added. Temperature and stirring conditions are maintained for two additional hours and the bulk of the reaction is allowed to reach 20 °C. After 20 h, the methanol is evaporated to dryness. The residue is dissolved in 500 ml of methylene chloride and 750 ml of water and shaked for 30 min. The organic phase is separated and the aqueous phase is extracted with 250 ml of methylene chloride. The organic phases are combined, filtered over celite, dried with anhydrous sodium sulphate and concentrated to dryness to yield 85 g of 9-deoxo-9a-aza-11,12-desoxy-9a-homoerythromycin A 11,12-hydrogenorthoborate.

IR (KBr)	ν_{max}= 3500, 2980, 2960, 1730, 1470, 1390, 1170, 1090, 1060 cm^{-1}
^{1}H-NMR ($CDCl_3$) (partial)	δ = 2.21 (NMe_2), 3.27 (OMe) ppm.
^{13}C-NMR ($CDCl_3$) (partial)	δ = 180.0 (C=O), 79.63 (C_{11}), 76.46 (C_{12}) 58.7 (C_{10}), 57.1 (C_9), 49.4 (OMe), 40.2 (NMe_2) ppm
^{11}B-NMR($CDCl_3$) TLC	δ = 9.9 ppm $\omega_{1/2}$= 200 Hz rf= 0.28 (petroleum ether : ethyl acetate: diethylamine 75: 25:10) developer: ethanol/vanillin (sulphuric acid)

- **Preparation of 9-deoxo-9a-aza-11,12-desoxy-9a-methyl-9a-homo-erythromycin A 11,12-hydrogenorthoborate.**

50 g of 9-deoxo-9a-aza-11,12-desoxy-9a-homoerythromycin A 11,12-hydrogenorthoborate are dissolved in 500 ml of chloroform, and subsequently a mixture of 5.5 ml of formic acid and 11.75 ml of aqueous 35-40% formaldehyde is added. The reaction mixture is heated under pressure for 14 hours and subsequently cooled down to 15-20°C. 500 ml of water are added and the mixture is taken to pH=4 by adding 20% sulphuric acid. The mixture is shaken for 15 min and the lower organic layer is separated. The alkaline aqueous phase is extracted with 2x100 ml methylene chloride. The organic phases are combined and filtered over celite, dried with anhydrous sodium sulfate and evaporated to dryness. The residue obtained is washed twice with 250 ml of ethyl ether, yielding a dry residue of 29 g of 9-deoxo-9a-aza-11,12-desoxy-9a-methyl-9a-homo-erythromycin A 11,12-hydrogenorthoborate.

IR (KBr)	ν_{max}= 3500, 1730, 1470, 1390, 1090, 1070 cm^{-1}
^{1}H-NMR ($CDCl_3$) (partial)	δ = 2.00 (NMe_2), 2.30 (NMe), 3.37 (OMe) ppm
^{13}C-NMR ($CDCl_3$) (partial)	δ = 179.9 (C=O), 79.40 (C_{11}), 77.09 (C_{12}), 68.84 (C_9), 64.08 (C_{10}), 49.36 (OMe), 40.18 (NMe_2), 34.39 (NMe) ppm
^{11}B-NMR($CDCl_3$)	δ= 10.1 ppm $\omega_{½}$= 180 Hz
m/e	M^+= 775.5
TLC	rf= 0.38 (petroleum ether : ethyl acetate: diethylamine 75:25:10) developer: ethanol/ vanillin (sulphuric acid)

- **Hydrolysis of 9-deoxo-9a-aza-11,12-desoxy-9a-methyl-9a-homo-erythromycin A 11,12-hydrogenorthoborate. Synthesis of 9-deoxo-9a-aza-9a-methyl-9a-homo-erythromycin A (Azithromycin).**

22 g of 9-deoxo-9a-aza-11,12-desoxy-9a-methyl-9a-homo-erythromycin A 11,12-hydrogenorthoborate are dissolved in 250 ml of acetonitrile to which 125 ml of water are subsequently added. 20% sulphuric acid is added to the mixture to take it to pH=2, and stirring is maintained for 30 min. The acidic solution is poured into a mixture of 350 ml of methylene chloride and 350 ml of water, inmediately adding 48% lime until pH=9. The mixture is shaken for 15 min and the lower organic phase is separated. The alkaline aqueous phase is extracted with 2x100 ml of methylene chloride. The combined organic phases are filtered over celite and evaporated to dryness. The residue is dissolved in 50 ml of ethanol and 60 ml of water are added over 30 min. Precipitation is allowed for 2 h, and the solid is collected by filtration and vacuum-dried at 40°C to yield 15 g of 9-deoxo-9a-aza-9a-methyl-9a-homo-erythromycin A (Azithromycin monohydrate).

IR (KBr)	ν_{max}= 3500, 3000, 2970, 1740, 1470, 1380, 1280, 1060 cm^{-1}
^{1}H-NMR ($CDCl_3$) (partial)	δ = 2.31 (NMe_2), 2.34 (NMe), 3.38 (OMe) ppm
^{13}C-NMR ($CDCl_3$) δ = (partial)	178.9 (C=O), 73.08 (C_{12}), 72.32 (C_{11}), 69.88 (C_9), 62.43 (C_{10}), 49.37 (OMe), 40.23 (NMe_2), 35.92 (NMe) ppm
m/e	M^+= 749.5
HPLC	corresponds according to *USP XXIII*
TLC	rf= 0.62 (petroleum ether : ethyl acetate : diethylamine 75:25:10) developer: ethanol/vanillin (sulphuric acid)

- **Preparation of non-crystalline 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A.**

5 g of crystalline azithromycin monohydrate are dissolved in 25 ml of *tert*-butanol heating at 30°C. This solution is filtered and solidified in a cooling bath. The solvent is sublimed at room temperature and 10^{-2}mm Hg. The solid obtained is dried (80 mm Hg /40 °C) to yield 5 g of non-crystalline azithromycin.

IR (KBr) ν_{max}= 3500, 1740, 1470, 1280, 1257 cm^{-1} (See Fig. 2)

^{1}H-NMR ($CDCl_3$), ^{13}C-NMR ($CDCl_3$), m/e, TLC and HPLC are identical to those of the previous example

% H_2O (K.F.)= 3.0 %

DSC = See **Fig. 3**

X-Ray Diffraction = See **Fig. 4**

Claims

1. A process for the preparation of 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A (Azithromycin) in its non-crystalline form **characterized by** the lyophilization of a solution of crystalline azithromycin monohydrate in aliphatic alcohols or cyclic ethers.

2. A process of claim 1 wherein the solvent used for lyophilization is *tert*-butanol.

3. A process of claim 1 wherein the solvent used for lyophilization is 1,4-dioxane.

4. A process for the preparation of 9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A (Azithromycin) in its non-crystalline form **characterized by**:

   ✓ Hydrolysis of 9-deoxo-9a-aza-11,12-desoxy-9a-methyl-9a-homo-erythromycin A 11,12-hydrogenorthoborate in an organic solvent (ethyl acetate, acetonitrile, methanol or ethanol) by the action of a dilute acid (sulphuric acid, hydrochloric acid, oxalic acid) at room temperature and at a pH range comprised between 2 and 4, to yield azitromycin monohydrate, and
   ✓ Lyophilization of the solution of azithromycin monohydrate in *tert*-butanol.

Patentansprüche

1. Verfahren zur Synthese von 9 - Desoxo - 9a - aza - 9a - methyl - 9a - homoerythromycin A (Azithromycin) in dessen nichtkristalliner Form,
   dadurch gekennzeichnet, dass eine Lösung des kristallinen Azithromycin · Monohydrats in aliphatischen Alkoholen oder cyclischen Ethern gefriergetrocknet (lyophilisiert) wird.

2. Verfahren nach Anspruch 1, bei dem das für die Gefriertrocknung verwendete Lösungsmittel in Form von *tert*-Butanol vorliegt.

3. Verfahren nach Anspruch 1, bei dem das für die Gefriertrocknung verwendete Lösungsmittel in Form von *tert*-Butanol vorliegt.

4. Verfahren zur Synthese von 9 - Desoxo - 9a - aza - 9a - methyl - 9a - homoerythromycin A (Azithromycin) in dessen nichtkristalliner Form,
   dadurch gekennzeichnet, dass

   ✓ das 11,12 - Hydrogenorthoborat von 9 - Desoxo - 9a - aza- 11,12 - desoxy - 9a - - methyl - 9a - homoerythromycin A in einem organischen Lösungsmittel (Ethylacetat, Acetonitril, Methanol oder Ethanol) durch die Aktivität einer verdünnten Säure (Schwe-(felsäure, Salzsäure oder Oxalsäure) bei Raumtemperatur und einem pH-Bereich zwischen 2 und 4 zur Gewinnung von Azithromycin·Monohydrat hydrolysiert wird, und
   ✓ die Lösung des Azithromycin·Monohydrats in *tert*-Butanol lyophilisiert wird.

Revendications

1. Procédé pour la préparation de la 9-désoxo-9a-aza-9a-méthyl-9a-homoérythromycine A (azithromycine) sous sa forme non-cristallisée, **caractérisé par** la lyophilisation d'une solution de monohydrate d'azithromycine cristallisé dans des alcools aliphatiques ou des éthers cycliques.

2. Procédé selon la revendication 1, dans lequel le solvant utilisé pour la lyophilisation est le *tert*-butanol.

3. Procédé selon la revendication 1, dans lequel le solvant utilisé pour la lyophilisation est le 1,4-dioxane.

4. Procédé pour la préparation de la 9-désoxo-9a-aza-9a-méthyl-9a-homoérythromycine A (azithromycine) sous sa forme non-cristallisée, **caractérisé par**

   ✓ l'hydrolyse du 11,12-hydrogénoorthoborate de 9-désoxo-9a-aza-11,12-désoxy-9a-méthyl-9a-homoé-

rythromycine A dans un solvant organique (acétate d'éthyle, acétonitrile, méthanol ou éthanol) par action d'un acide dilué (acide sulfurique, acide chlorhydrique, acide oxalique) à la température ambiante et dans un intervalle de pH compris entre 2 et 4, pour obtenir le monohydrate d'azithromycine et

✓ la lyophilisation de la solution de monohydrate d'azithromycine dans du *tert*-butanol.

Figure 1 : Synthesis of Azithromycin

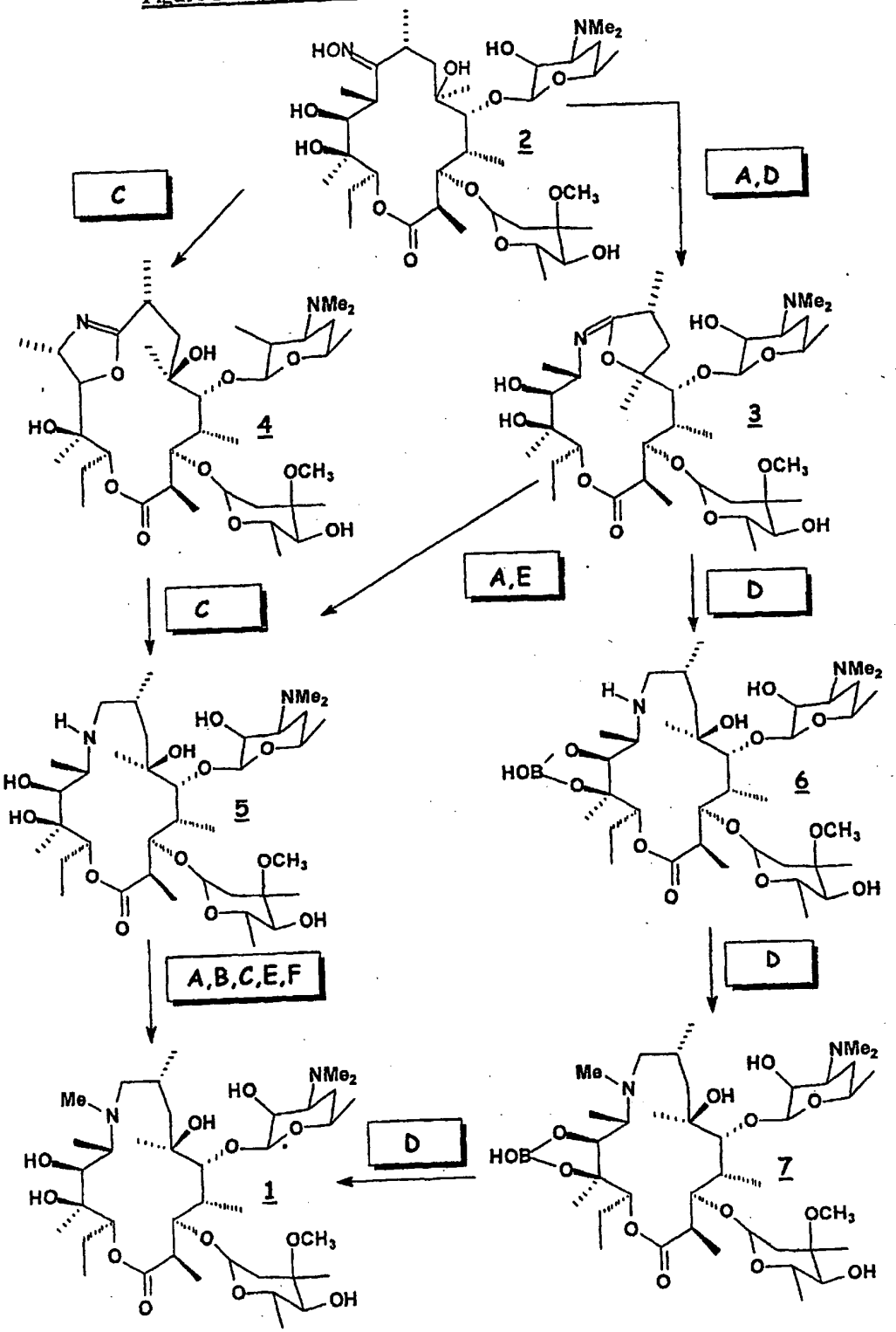
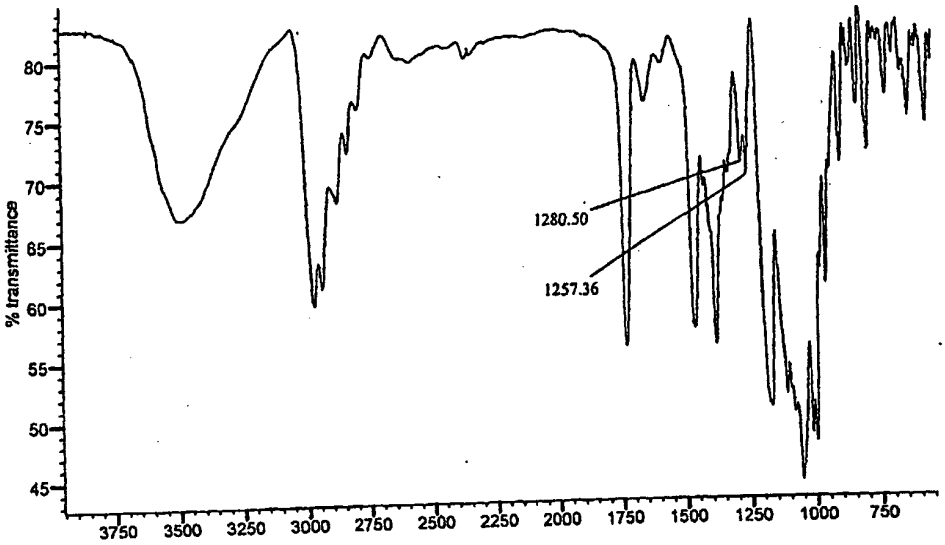


Figure 2

Non crystalline Azithromycin



Crystalline Azithromycin dihydrate

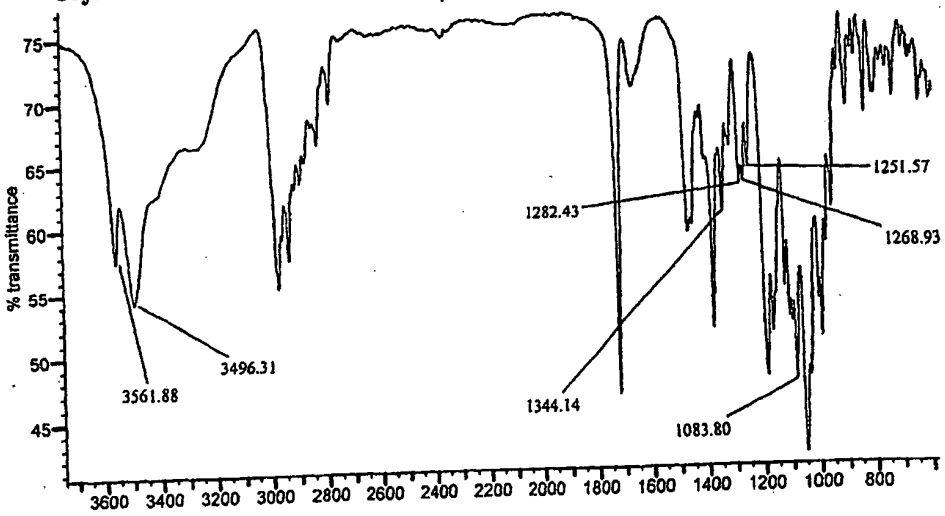
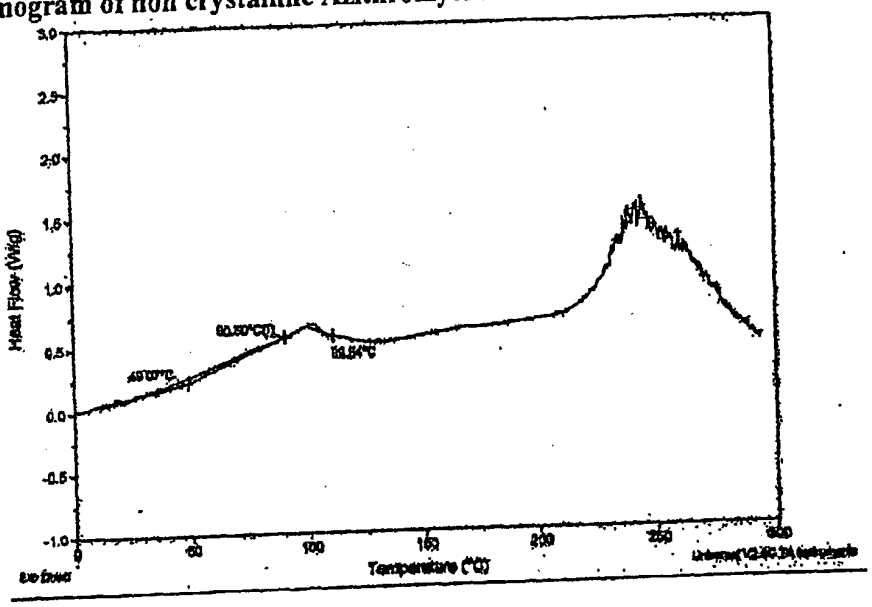


Figure 3

Thermogram of non crystalline Azithromycin



Thermogram of crystalline Azithromycin dihydrate

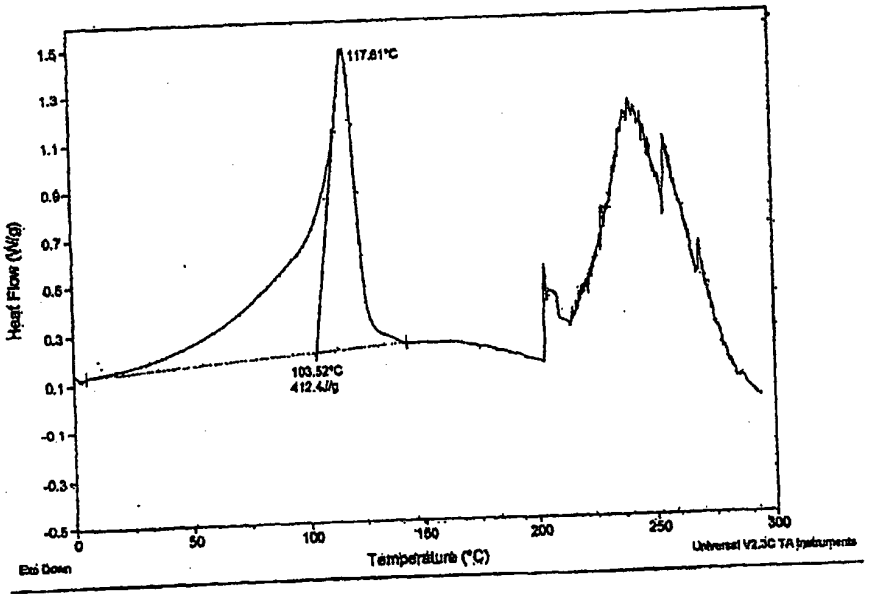
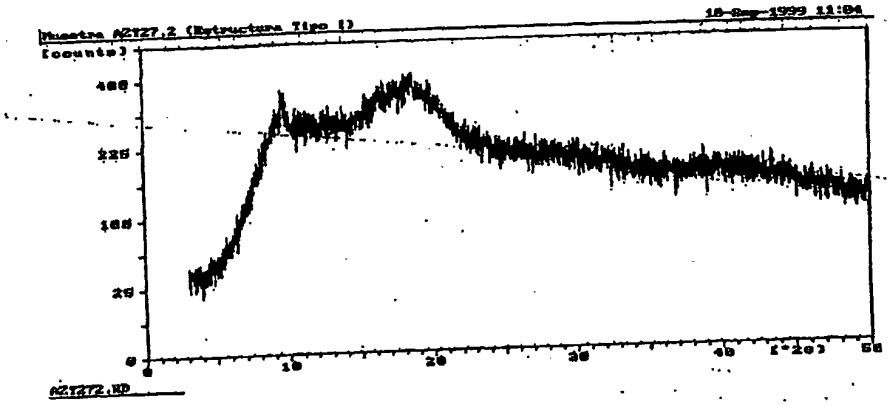


Figure 4

Non crystalline Azithromycin



Crystalline Azithromycin dihydrate